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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JOHANNES M. VAN DEN BRINK, MARIANNE K. HARBOE, STEEN G. PETERSEN, and HENRIK RAHBEK-NIELSEN

Appeal 2010-001320
Application 10/518,414
Technology Center 1600

Decided: February 3, 2010

Before DEMETRA J. MILLS, ERIC GRIMES, and LORA M. GREEN, *Administrative Patent Judges*.

GREEN, *Administrative Patent Judge.*

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the Examiner's final rejection of claims 1-5, 8-15, 17-19, 22, and 23. We have jurisdiction under 35 U.S.C. § 6(b).

STATEMENT OF THE CASE

Claims 1 and 17 are representative of the claims on appeal, and read as follows:

1. A process for producing an isolated polynucleotide sequence encoding a modified polypeptide comprising: i) modifying a polynucleotide sequence that comprises a DNA sequence encoding a polypeptide comprising an aspartic protease amino acid sequence to encode an extra polypeptide N-X-T glycosylation site in the aspartic protease amino acid sequence; and ii) isolating the polynucleotide sequence resulting from step (i) which isolated polynucleotide sequence encodes the modified polypeptide.

17. An isolated polypeptide exhibiting aspartic protease activity comprising a N-X-T glycosylation site, wherein the aspartic protease is a chymosin.

The Examiner relies on the following evidence:

Budtz	US 5,800,849	Sep. 1, 1998
Harboe	US 6,127,142	Oct. 3, 2000

Kasturi et al., *Regulation of N-linked core glycosylation: use of a site-directed mutagenesis approach to identify Asn-Xaa-Ser/Thr sequons that are poor oligosaccharide acceptors*, 323 J. BIOCHEMISTRY 415-419 (1997).

Korman et al., *Cloning, characterization, and expression of two α-amylase genes from Aspergillus niger var. awamori*, 17 CURR. GENET. 203-212 (1990).

The following grounds of rejection are before us for review:

I. Claims 17-19 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Harboe.

II. Claims 1-5, 8, 12-15, 17-19, and 22 stand rejected under 35 U.S.C. § 103(a) over the combination of Budtz, Kasturi, and Harboe.

III. Claims 9-11 and 23 stand rejected under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Budtz, Kasturi, and Harboe as further combined with Korman.

We reverse.

ISSUE (Rejection I)

The Examiner finds that claims 17-19 are anticipated by Harboe.

Appellants contend that Harboe relates to a method for deglycosylating an aspartic protease from *Rhizomucor miehei* (EC 3.4.23.23 Mucor rennin), which is not a chymosin (EC 3.4.23.4).

Thus, the issue on appeal is: Have Appellants demonstrated that the Examiner erred in finding that the aspartic protease from *Rhizomucor miehei* (EC 3.4.23.23 Mucor rennin) is a member of the chymosin (EC 3.4.23.4) subfamily of aspartic proteases?

FINDINGS OF FACT

FF1 The Specification teaches that "[e]nzymatic coagulation of milk by milk-clotting enzymes, such as chymosin and pepsin, is obviously one of the most important processes in the manufacture of cheeses." (Spec. 1.)

FF2 According to the Specification, "[c]hymosin (EC 3.4.23.4) and pepsin (EC 3.4.23.1), the milk clotting enzymes of the mammalian stomach, are aspartic proteases that are produced naturally in the gastric mucosal cells of several mammalian species including ruminant species, porcine species, primate species and ungulate species." (*Id.* at 2.)

FF3 The Specification teaches that "[c]hymosin is furthermore featured by having two N-X-S glycosylation sites which however are poorly glycosylated." (*Id.* at 3.)

FF4 According to the Specification, the issue addressed by the invention is "to get increased production yields of a recombinantly produced aspartic protease, such as e.g. chymosin, without substantially influencing the aspartic protease enzyme properties as such." (*Id.* at 5.)

FF5 The Specification teaches that it has been discovered "that the recombinant production capacity of an aspartic protease, such as e.g. chymosin, can be increased by an alteration of a specific glycosylation site . . . . by incorporating a new N-X-T glycosylation site into a polypeptide comprising an aspartic protease amino acid sequence." (*Id.*)

FF6 The Examiner rejects claims 17-19 under 35 U.S.C. § 102(b) as being anticipated by Harboe (Ans. 4).

FF7 The Examiner finds that Harboe "teaches an aspartic protease used for clotting milk (column 1, lines 34-40)," which the Examiner finds "should have an activity ratio similar to bovine chymosin (column 6, lines 38-41), the implication being that bovine chymosin is acceptable to use as the protease." (*Id.*)

FF8 The Examiner finds further that the "modified aspartic protease has a glycosylation site, which can be the sequence N-X-T (column 3, lines 38-41)." (*Id.*)

FF9 The Examiner finds further that Harboe teaches the enzyme Hannilase™, "which is a member of the chymosin family." (*Id.* at 9.)

FF10 Harboe relates "to aspartic proteases derived from *Rhizomucor miehei* and having improved milk clotting activity." (Harboe, col. 1, ll. 9-10.)

FF11 Harboe teaches that milk clotting enzymes of microbial origin are in commercial use in the dairy industry, and include proteases natively produced by the zygomycete filamentous fungal species *Rhizomucor miehei* (*id.* at col. 1, ll. 27-30).

FF12 Harboe notes that "the coagulant produced natively by *Rhizomucor miehei* has three possible N-linked glycosylation sites i.e. sites having the sequence Asn-X-Thr/Ser at Asn^{79}, Asn^{188} and Asn^{313}." (*Id.* at col. 3, ll. 39-41.)

FF13 Harboe teaches:

> It has now surprisingly been found that homologous *Rhizomucor miehei* aspartic protease . . . acquires a significantly enhanced milk clotting activity when it is deglycosylated, and furthermore, that the milk clotting activity of heterologous *Rhizomucor miehei* aspartic protease as produced in *Aspergillus oryzae*, contrary to the above disclosures by a manufacturer hereof, is enhanced significantly by deglycosylation.

(*Id.* at col. 2, ll. 46-54.)

FF14 Harboe teaches that the glycosylated coagulant (i.e., the aspartic protease) may be deglycosylated enzymatically or chemically (*id.* at col. 3, ll. 23-33).

FF15 Harboe teaches:

> An important characteristic for a milk clotting enzyme is that its MCA does not depend to any significant degree on the particular pH in the milk. This may e.g. be expressed as activity ratio at 2 pH values within the relevant pH range which is 6-7. Thus, as an example, a suitable milk clotting enzyme should ideally have an activity ratio similar to or close to that of

> pure calf chymosin for milk clotting activity at two different pH values such as 6.0/6.5 or 6.5/7.0. Pure (100%) calf chymosin has a pH 6.0/6.5 activity ratio of 0.85.
>
> Thus, it was found during the experimentation leading to the present invention that treatment of certain *Rhizomucor miehei* aspartic proteases having a relatively high pH dependency (i.e. activity ratios above that of calf chymosin) with Endo H reduced the pH 6.0/6.5 or the 6.5/7.0 activity ratios to values closer to that of calf chymosin. The order of this reduction was typically (pH 6.0/6.5) in the range of 10-30%, e.g. from 1,34 [sic] to 1.12 (17% reduction) for Modilase® or from 1.02 to 0.81 (21% reduction) for Hannilase®. Accordingly, the method according to the invention is preferably one wherein the resulting deglycosylated enzyme has a low pH dependency as defined above.

(*Id.* at col. 6, ll. 34-54.)

FF16 Harboe uses two commercial liquid *Rhizomucor miehei* coagulant products of the product series MICROLANTT™, which are "produced by submerged fermentation of a selected strain of *Rhizomucor miehei* naturally producing the active milk clotting protease (EC 3.4.23.23)." (*Id.* at col. 8, ll. 17-23.) Harboe teaches that the "enzyme-containing standardized commercial products are marketed under the trade names Hannilase® and Modilase®." (*Id.* at col. 8, ll. 23-27.)

PRINCIPLES OF LAW

"It is well settled that a claim is anticipated if each and every limitation is found either expressly or inherently in a single prior art reference." *Celeritas Techs. Ltd. v. Rockwell Int'l Corp.*, 150 F.3d 1354, 1361 (Fed. Cir. 1998).

ANALYSIS

Appellants argue that Harboe "relates to a method for deglycosylating an aspartic protease from *Rhizomucor miehei* (EC 3.4.23.23 Mucor rennin), which is not a chymosin (EC 3.4.23.4)." (App. Br. 10.) Appellants argue further that the Hannilase™ cited in Example 1 of Harboe "is not a member of the chymosin family," but is in fact a *Rhizomucor* protease. (Reply Br. 5.)

We agree with Appellants. The Specification notes that chymosin (EC 3.4.23.4), a subfamily of the family of aspartic proteases, is produced naturally in the gastric mucosal cells of several mammalian species including ruminant species, porcine species, primate species and ungulate species. Harboe teaches an aspartic protease produced by the zygomycete filamentous fungal species *Rhizomucor miehei*. In Example 1, relied upon by the Examiner, uses two commercial liquid *Rhizomucor miehei* coagulant products of the product series MICROLANTT™, which are "produced by submerged fermentation of a selected strain of *Rhizomucor miehei* naturally producing the active milk clotting protease (EC 3.4.23.23)," and the "enzyme-containing standardized commercial products are marketed under the trade names Hannilase® and Modilase®." (FF16.)

The Examiner has not provided any evidence demonstrating that the ordinary artisan would consider the *Rhizomucor miehei* aspartic proteases (EC 3.4.23.23), such as Hannilase®, as members of the chymosin (EC 3.4.23.4) subfamily of aspartic proteases, which the Specification teaches are produced naturally in the gastric mucosal cells of several mammalian

species including ruminant species, porcine species, primate species and ungulate species.

The Examiner also relies on the teaching of Harboe that the aspartic acid protease used for clotting milk should have an activity ratio similar to bovine chymosin, concluding that bovine chymosin is acceptable to use as the protease. However, while that may be teaching that chymosin may be used in the clotting process, it does not imply that *Rhizomucor miehei* aspartic protease is a member of the chymosin subfamily of aspartic proteases.

CONCLUSION OF LAW

We find that Appellants have demonstrated that the Examiner erred in finding that the aspartic protease from *Rhizomucor miehei* (EC 3.4.23.23 Mucor rennin) is a member of the chymosin (EC 3.4.23.4) subfamily of aspartic proteases.

We thus reverse the rejection of claims 17-19 under 35 U.S.C. § 102(b) as being anticipated by Harboe.

ISSUE (Rejections II and III)

The Examiner concludes that claims 1-5, 8, 12-15, 17-19, and 22 are rendered obvious by the combination of Budtz, Kasturi, and Harboe.

Appellants contend that the Examiner has engaged in improper hindsight, and has thus failed to set forth a prima facie case of obviousness.

Thus, the issue on appeal is: Have Appellants demonstrated that the Examiner engaged in improper hindsight to combine the teachings of Budtz,

Kasturi, and Harboe, and thus has failed to establish a prima facie case of obviousness?

FINDINGS OF FACT

FF17 The Examiner rejects claims 1-5, 8, 12-15, 17-19, and 22 under 35 U.S.C. § 103(a) over the combination of Budtz, Kasturi, and Harboe (Ans. 5).

FF18 The Examiner finds that Budtz "teaches a process for producing cheese by adding an aspartic protease to clot milk." (*Id.*)

FF19 The Examiner finds that the process taught by Budtz "includes isolating a DNA sequence encoding a bovine prochymosin and transforming it into *Aspergillis* (column 1, lines 37-40, 54, and 55-56)," wherein the "prochymosin has an N-bound glycosylation site (column 3, lines 11-15)." (*Id.*)

FF20 Budtz notes that it is necessary to coagulate milk in the production of cheese in order to separate the casein from the whey (Budtz, col. 1, ll. 13-14). Budtz teaches that "the glycosylation of . . . aspartic protease can give an increase in cheese yield of 0.2% compared with the native enzyme." (*Id.* at col. 1, ll. 25-27.) The aspartic protease may be a chymosin or a microbial aspartic protease, such as that produced by *Rhizomucor miehei* (*id.* at col. 1, ll. 36-54).

FF21 Budtz teaches that the glycosylation of the recombinant aspartic protease is higher than that of the native form (*id.* at col. 2, ll. 57-67).

FF22 The Examiner notes that Budtz "does not teach the glycosylation site being N-X-T." (Ans. 6.)

FF23 The Examiner relies on Kasturi for teaching an "N-linked glycosylation protein," wherein the "glycosylation site is N-X-T." The Examiner further finds that the N-X-T glycosylation site "can be mutated by site directed mutagenesis, and therefore be an artificial linker." (*Id.*)

FF24 Kasturi teaches that "N-linked glycosylation usually occurs at the sequon Asn-Xaa-Ser/Thr, where Xaa is any amino acid residue except Pro." (Kasturi, Abstract.) Kasturi notes that many such sequons are inefficiently glycosylated, or not at all (*id.*).

FF25 Kasturi thus used site directed mutagenesis to "examine how the Xaa and hydroxy (Ser/Thr) amino acid residues in sequons influence core-glycosylation efficiency." (*Id.*)

FF26 Specifically, Kasturi used the "rabies virus glycoprotein (RGP) as a model system to examine systematically the impact of local amino acid residues on core-glycosylation efficiency." (*Id.* at 415, second column.)

FF27 The Examiner notes that Kasturi does not teach an "N-linked aspartic protease." (Ans. 6.)

FF28 Harboe is relied upon as set forth in the anticipation rejection (*see* FF 7-8).

FF29 The Examiner concludes:

> The ordinary skilled artisan, desiring to use a N-X-T glycosylation site in chymosin would have been motivated to combine the teachings of [Budtz] of process of isolating a DNA sequence encoding a N-glycosylated bovine prochymosin and transforming it into *Aspergillus* with the teachings of Kasturi et al of an N-X-T linked protein and of [Harboe], of a modified aspartic protease that has a glycosylation site of N-X-T, because Kasturi et al teach that N-linked glycosylation usually occurs at

> N-X-S/T sites, and N-glycosylation profoundly affects a protein's expression and function.

(Ans. 6.)

FF30 The Examiner rejects claims 9-11 and 23 under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Budtz, Kasturi, and Harboe as further combined with Korman (Ans. 7).

FF31 Korman is cited for teaching "a vector with a gene fusion of alpha-amylase and bovine prochymosin transformed into *Aspergillus awamori*." (*Id.* at 8.)

PRINCIPLES OF LAW

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) secondary considerations of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

While the analysis under 35 U.S.C. § 103 allows flexibility in determining whether a claimed invention would have been obvious, *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007), it still requires showing that "there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *Id.* "We must still be careful not to allow hindsight reconstruction of references to reach the claimed invention without any explanation as to how or why the references would be combined to produce the claimed invention." *Innogenetics, N.V. v. Abbott Labs.*, 512 F.3d 1363, 1374 n.3 (Fed. Cir. 2008).

ANALYSIS

Appellants argue that the rejection is "based on improper hindsight-based reasoning." (App. Br. 15.) Appellants argue that the present invention is drawn to providing "a method for more efficiently producing aspartic protease in a host organism," asserting that the combination of references cited by the Examiner "would in no way would have suggested addressing that problem by modifying the polynucleotide sequence to encode an (extra) N-X-T glycosylation site in the aspartic protease amino acid sequence, and subsequently expressing the sequence." (*Id.* at 12.)

Appellants argue that, in response to their request to the Examiner to state a reason for combining the references, the Examiner stated that the "motivation is that Kasturi et al. teach that N-linked glycosylation usually occurs at N-X-S/T sites, and N-glycosylation profoundly affects a protein's expression and function." (*Id.* at 12 (quoting Advisory Action dated January 28, 2009).)

Appellants assert that the Examiner's reasoning is flawed (App. Br. 13). Appellants argue that Budtz teaches that increased glycosylation was found to be advantageous, but did not teach that the increased glycosylation site was due to the site being N-X-T, but due to expression of the gene in Aspergillus/Trichoderma host cells (*id.*). Kasturi, Appellants argue, compares the impact of the Xaa residue in Asn-Xaa-Ser with that of Asn-Xaa-Thr to define protein sequences that control N-linked glycosylation, using rabies virus glycoprotein as the primary model (*id.*). Harboe, Appellants assert, teaches that decreased glycosylation resulted in improved clotting activity in aspartic proteases derived from *Rhizomucor miehei* (*id.* at

13-14). Appellants thus assert that the Examiner's reasoning is not consistent with the teachings of Harboe which teaches "significantly enhanced clotting activity associated with deglycosylated aspartic protease." (*Id.* at 15.)

We agree with Appellants that the Examiner has engaged in improper hindsight to arrive at the claimed invention. Budtz teaches that producing an aspartic protease recombinantly increases glycosylation of the protein, thus increasing can give an increase in cheese yield of 0.2% compared with the native enzyme. Budtz does not teach or suggest engineering an extra glycosylation site into the aspartic protease. Harboe, on the other hand, teaches that homologous, as well as heterologous, *Rhizomucor miehei* aspartic protease acquires a significantly enhanced milk clotting activity (FF13). Kasturi teaches that N-linked glycosylation usually occurs at Asn-Xaa-Ser/Thr, and examined how the Xaa and hydroxyl (Ser/Thr) amino acid residues in sequons influence core-glycosylation efficiency.

Thus, while the Examiner concludes that ordinary skilled artisan, desiring to engineer a N-X-T glycosylation site in chymosin would have been motivated to combine the teachings of Budtz, Kasturi, and Harboe, she does not provide a clear explanation or reason to as to why the ordinary artisan would have wanted to engineer an extra site into chymosin, especially given the conflicting teachings of Budtz and Harboe. The Examiner appears to be relying on Kasturi for teaching that an N-X-T site may be used as a linker (*see* FF23), but the Examiner does not point to, nor can we find, that teaching in Kasturi. Nor does the Examiner provide any reason as to why the ordinary artisan would want to have an N-linked

glycosylation site as a linker. Thus, the Examiner has failed to establish a prima facie case of obviousness.

As to the rejection of claims 9-11 and 23 over the combination of Budtz, Kasturi, and Harboe as further combined with Korman, we conclude that Korman does not remedy the deficiencies of the combination of Budtz, Kasturi, and Harboe. Thus, the Examiner has failed to set forth a prima facie case of obviousness in this rejection as well.

CONCLUSIONS OF LAW

We conclude that Appellants have demonstrated that the Examiner engaged in improper hindsight to combine the teachings of Budtz, Kasturi, and Harboe, and thus has failed to establish a prima facie case of obviousness.

We thus reverse the rejection of claims 1-5, 8, 12-15, 17-19, and 22 under 35 U.S.C. § 103(a) over the combination of Budtz, Kasturi, and Harboe.

As Korman does not remedy the deficiencies of the combination of Budtz, Kasturi, and Harboe, we also reverse the rejection of claims 9-11 and 23 under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Budtz, Kasturi, and Harboe as further combined with Korman.

REVERSED

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